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EXUDATE FLAVONOIDS OF *INULA VISCOSA*

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Key Word Index—*Inula viscosa*; Asteraceae; exudate flavonoids; aglycones.

Abstract—Aerial parts of *Inula viscosa* exhibit a resinous exudate of terpenoid nature, containing flavonoid aglycones. Nine flavonoids are reported in addition to 13 previously known components. The study of samples collected in France, Spain and Turkey indicates the existence of a qualitatively constant exudate flavonoid profile in this species.

INTRODUCTION

Some years ago, a detailed study on *Inula viscosa* (L.) Ait. (Asteraceae, Inuleae) revealed the presence of some 16 flavonoid aglycones, found in the acetone extract of ground aerial parts [1]. As the species name *viscosa* of this perennial Mediterranean compositae indicates, its aerial parts are sticky with a resinous material. This exudate consists of terpenoids, such as the sesquiterpene acids described in refs [2, 3]. We assumed that the flavonoid aglycones are dissolved in the resinous material, as this is a well known feature of many Asteraceae [4] and also of plants from other families [5]. The localization of flavonoids was not considered in the above report, as an acetone extract of ground material was analysed [1].

RESULTS AND DISCUSSION

In the acetone-wash of *Inula viscosa* aerial parts, a series of flavonoid aglycones was encountered. In material collected in southern France and Spain, we found the flavones apigenin, scutellarein 6-methyl ether (hispidulin), luteolin, 6-methoxyluteolin (nepetin); the flavonols kaempferol 3-methyl ether (isokaempferide), kaempferol 7-methyl ether (rhamnocitrin), 6-methoxykaempferol, quercetin and its 3-methyl, 7-methyl (rhamnetin), 3′-methyl (isorhamnetin) and 3,3′-dimethyl ethers, quercetagetin 3,6-dimethyl ether (axillarin) and quercetagetin 6,3′-dimethyl ether (spinacetin); the flavanones naringenin 7-methyl ether (sakuranetin). eriodictyol and eriodictyol 7-methyl ether; the dihydroflavonols aromadendrin 3-acetate, aromadendrin 7-methyl ether, taxifolin 3-acetate, taxifolin 7-methyl ether, and taxifolin 7-methyl ether-3-acetate (aromadendrin = dihydrokaempferol, taxifolin = dihydroquercetin). Except for taxifolin 7-methyl ether, all these flavonoids were identified unambiguously by direct comparisons with available authentic samples. Kaempferol 7-methyl ether and the 3-methyl, 7-methyl and 3,3′-dimethyl derivatives of quercetin as well as taxifolin 7-methyl ether-3-acetate were obtained in crystalline form. These products were further charaterized by their mps as well as their UV and mass spectral data, which agreed with those reported in literature (for taxifolin 7-methyl ether and taxifolin 7-methyl ether-3-acetate; see ref. [1]). The ^{13}C NMR data of taxifolin 7-methyl ether and taxifolin 7-methyl ether-3-acetate according to our knowledge have not been reported previously and are presented in Table 1 along with those of a taxifolin standard.

Table 1. ^{13}C NMR data of taxifolin, taxifolin 7-Me and taxifolin 7-ME-3-Ac in acetone

C	Taxifolin	Taxifolin 7-ME	Taxifolin 7-Me-3-Ac
2	83.9	84.5	81.4
3	72.5	73.1	72.4
4	197.6	198.6	192.8
5	164.3	164.5	164.2
6	96.4	95.6	95.4
7	167.2	169.2	168.8
8	95.4	94.6	94.4
9	163.5	163.9	163.0
10	101.0	102.1	101.9
1′	129.1	129.6	127.5
2′	115.1[a]	115.7[a]	114.8[a]
3′	145.1	145.7	145.3
4′	145.9	146.5	146.4
5′	115.2[a]	115.8[a]	115.3[a]
6′	120.2	120.8	120.0
OMe	—	56.3	55.8
MeCo	—	—	19.6
MeCO	—	—	168.8

[a]Signals interchangeable.

Aromadendrin 7-methyl ether and taxifolin 7-methyl-ether-3-acetate appeared on polyamide TLC, after spraying with Naturstoffreagenz A (NA) as spots with somewhat unusual greenish and yellow fluorescence in UV_{366}, while eriodictyol and in particular its 7-methyl ether were distinguished by reddish fluorescence. One spot attracted our special attention, due to its exceptional colour behaviour. It appeared, with almost the same R_f as kaempferol 7-methyl ether, as a faint spot that turned

grey with NA. The centre of concentrated spots was dark in UV and white in daylight. After some exposure to UV, or to daylight for several hours, this spot became a conspicuous apricot colour. Isolation of this compound in a pure state was extremely difficult, but was finally achieved by polyamide prep. TLC and the structure elucidated by NMR spectroscopic studies (cf. Table 1) as taxifolin 7-methyl ether. It was identical in every respect with the product obtained on acid hydrolysis of taxifolin 7-methyl ether 3-acetate.

In the earlier study on flavonoid aglycones of *Inula viscosa* [1], no attention was paid to the fact that these products are accumulated externally, dissolved in the terpenoid leaf and stem resin. The flavonoid pattern these authors reported for plant collected near Alicante in Spain is slightly different from what we found in bulk material from Narbonne-Plage, France and Vinaroz, Spain. Aromadendrin 7-methyl ether appeared to be the dominant flavonol in the plants from Vinaroz, whereas those from Narbonne-Plage produced more taxifolin 7-methyl ether-3-acetate; no other significant differences were noticed. We did not find the earlier reported [1] flavonoids apigenin 7-methyl ether, naringenin and aromadendrin 7-methyl ether-3-acetate, but this may well be a matter of concentration. Also no marker was available for aromadendrin 7-methyl ether-3-acetate. On the other hand, we found nine aglycones that had not been mentioned in [1], including eriodicytol and its 7-methyl ether and the two methyl derivatives of quercetagetin. A sample we collected near Alicante was very poor in total flavonoid production, but the aglycones appeared to be the same. In contrast, a sample from La Mancha (Spain) was found to be extrememly rich in exudate flavonoids. We also had a chance to analyse a sample collected near Antalya in Turkey, the flavonoid profile of which was very similar to that from La Mancha, but it contained considerably lower amounts of kaempferol 7-methyl ether, quercetin 7-methyl ether and aromadendrin 7-methyl ether. Qualitatively, the flavonoid profiles of the five samples studied were more or less constant, although three of them were collected in Spain, one in France and one in Turkey.

Flavonoid aglycones have also been reported from *Inula cappa*, collected in Assam, India [6]. This species exhibits three flavonoids with unusual B-ring substitution. They were found in a chloroform extract of above ground parts; they are hence assumed to be exudate constituents in this species, too. The same is probably true for the sesquiterpenoids reported from several *Inula* species.

EXPERIMENTAL

Bulk portions of *Inula viscosa* for flavonoid isolation were collected by E.W., (a) near Narbonne-Plage in southern France in August 1985 and (b) near Vinaroz in Spain in August 1989. Samples for comparison of flavonoid profiles originated in Alicante and in La Mancha in southern Spain (September 1988) and in the vicinity of Alanya, Turkey (April 1989). The exudate was washed from aerial parts by rinsing with Me_2CO. The resinous material obtained from the two bulk collections was passed over Sephadex LH-20 to separate flavonoids from terpenoids. TLC analysis of Sephadex frs revealed only minor quantitative differences between the two bulk samples. Corresponding frs were, therefore, combined and some subjected to CC on acetylated polyamide SC-6. Elution was with toluene and increasing amounts of MeCOEt and MeOH. Taxifolin 7-methyl ether -3-acetate was isolated from relevant frs by prep. TLC on plastic sheets precoated with polyamide 11 (Macherey-Nagel, Duren), developed in toluene–dioxan–MeOH (16:3:3). TLC controls were on polyamide DC-11 (toluene–petrol 5:7; MeCOEt–MeOH, 12:6.2:1 and toluene–dioxan–MeOH, 8:1:1) and on silica (toluene–dioxan–HOAc, 18.5:1). NA was used as spray reagent. Flavonoid identification was by direct comparison with markers, supported by mp, UV and MS data. Most markers were available in E.W.s lab, some originate from [1]. Taxifolin 7-methyl ether was identified by spectroscopic studies (cf. Table 1). ^{13}C NMR spectra were run in d_6-Me_2CO at 50 MHz. MS were obtained at 70 eV.

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